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(54)【発明の名称】 アポトーシス誘導剤

(57)【要約】

【解決手段】 TNF- α 及びIL-4を有効成分とするアポトーシス誘導剤。

【効果】 本発明アポトーシス誘導剤は、TNF- α 又はIL-4を単独で用いた場合に比べ、アポトーシス誘導効果が相乗的に増強され、副作用の少ない制癌剤、慢性関節リウマチ治療剤、自己免疫疾患治療剤、肝臓、肝臓等の肝疾患治療剤等として使用できる。

【特許請求の範囲】

【請求項1】 TNF- α 及びIL-4を有効成分とするアポトーシス誘導剤。

【請求項2】 悪性腫瘍又は慢性関節リウマチの予防・治療薬である請求項1記載のアポトーシス誘導剤。

【発明の詳細な説明】

【0001】

【発明の属する技術分野】 本発明はアポトーシス誘導剤に関するものである。

【0002】

【従来の技術】 アポトーシスはプログラムされた細胞死の一形態であり、古典的細胞死(ネクローシス)と対比されるものである。アポトーシスは生化学上の種々の条件下に起こり、その形態学的特徴として、周囲の細胞との接触の欠乏、細胞質の濃縮化、エンドスクレアーゼの活性化に関連したクロマチンの凝縮及び核膜崩壊、核の分断、細胞表面の微絨毛の消失、細胞表面の平滑化(細胞表面の水形成: membrane blebbing)及びエンドヌクレアーゼによるDNAの断片化が観察され、アポトーシス細胞の最終断片が脱落する細胞により貪食される機構として働いている (Bovall, E. and Wyllie, A. H., Immunology Today, 7 (4), 115-119 (1986); Science, 245, 301-305 (1989)) 。

【0003】 アポトーシスは正常な発生・分化に不可欠な生理的細胞死であり、正常な生体組織の細胞回転などにおいて個々の細胞に起こっているが、癌性腫瘍、白血病、増殖性皮膚疾患、慢性関節リウマチ、自己免疫疾患等の疾患においては、アポトーシスが過剰に抑制され、その結果、細胞に機能障害が生じると考えられている。例えば、ワタナベ・フクナガからはMR L1 p r / l p rマウスにおいては、アポトーシスに相当するFas分子に異常があり、胸腺における自己反応性T細胞のネガティブセレクション(アポトーシス)機構がうまく作動せず、その結果自己免疫疾患が発症すると示唆している (Watanabe-Fukunaga, K., et al., Nature, 356, 314-317 (1992)) 。また、慢性肝炎が肝硬変、肝臓に移行していく過程では、アポトーシスは抑制状態にあり、これがサイトキニンシット細胞による肝細胞の破壊に続く増殖化、肝硬変へと進展するものと考えられている。従って、斯かる疾患に罹患する細胞のアポトーシスを誘導する物質は、当該疾患の予防・治療薬として有用である。

【0004】 従来、蛋白質合成阻害剤であるシクロヘキシミドやRNA合成阻害剤であるアクチノマイシンD、腫瘍細胞死因子(以下、「TNF- α 」)という) やリンホトキニン(LT)等のサイトカイン類にアポトーシスの誘導作用があることが報告され (Martin, S. J., et al., J. Immunol., 145, 1859-1867 (1990); Strelow, A., et al., J. Exp. Med., 192, 601-611 (2000))、また最近ではインターロイキン4 (以下、「IL-4」)という)

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に、ヒト血球や好細胞に対してアポトーシス誘導作用があることが報告されている (J. Immunol., 148(6), 1812-1816 (1992); J. Allergy Clin. Immunol., 102(6 Pt. 1), 1013-1020 (1998)) 。

【0005】 しかし、これまでに知られている物質は、アポトーシス誘導活性や副作用の点から充分なものではなく、アポトーシス誘導活性が高く且つ安全性の高いアポトーシス誘導剤が求められていた。

【0006】

【発明が解決しようとする課題】 本発明は、有効性が高く且つ安全性の高いアポトーシス誘導剤を提供することを目的とする。

【0007】

【課題を解決するための手段】 本発明は、斯かる課題に鑑み、アポトーシス誘導活性を有する物質について鋭意研究した結果、TNF- α とIL-4を併用した場合に、未分化細胞や前駆体細胞に対してそれぞれが有するアポトーシス誘導効果が相乗的に増強され、アポトーシスの過剰抑制に伴う疾患の予防・治療薬として有用であることを見出し、本発明を完成した。

【0008】 すなわち、本発明は、TNF- α 及びIL-4を有効成分とするアポトーシス誘導剤を提供するものである。

【0009】

【発明の発明の形態】 本発明のアポトーシス誘導剤は、TNF- α とIL-4を有効成分とするものであるが、ここで、TNF- α とは、炎症を過剰に生体防御機構を中心に、抗腫瘍作用、破骨作用、細胞への脂質の取り込み作用、インターロイキン-1やコロニー刺激因子の生産誘導作用等、多様な生物活性を示す分子重さ17 kDaのポリペプチドであり、IL-4とは、広い範囲の免疫細胞刺激作用(B細胞の形質細胞への分化、T細胞の分化増殖)を中心に、抗腫瘍作用、I型アレルギー誘導作用、抗炎症作用等を有するサイトカインの一種である。これらTNF- α 及びIL-4には前述したように、アポトーシス誘導作用があることが報告されているが、TNF- α とIL-4を併用した場合に、アポトーシス誘導効果が相乗的に増強されることは全く予測することができなかったことである。

【0010】 本発明のアポトーシス誘導剤に用いられるTNF- α 及びIL-4としては、それぞれTNF- α 及びIL-4としての活性を有する、天然型或いは遺伝子組換えにより産生された組換え体の何れもが含まれる。

【0011】 天然型のTNF- α は、例えば、センダイウイルス(Sendai Virus) 刺戟ヒトBリンパ母細胞株BA13 L-1などの底物の細胞株の培養上清より、アフリニティークロマトグラフィーやHPLCなどの既知方法に従い精製することにより得ることができ、遺伝子の組換えによって得られるTNF- α は、既知の遺伝子を用い

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和、慢性関節リウマチ治療剤、自己免疫疾患治療剤、肝炎、肝硬変等の肝疾患治療剤等として使用できる。

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(21)Application number : 2000-318464 (71)Applicant : HIROHATA TOSHINARI
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(54) APOPTOSIS-INDUCING AGENT

(57)Abstract:

PROBLEM TO BE SOLVED: To obtain an apoptosis-inducing agent consisting mainly of TNF- α and IL-4 as active ingredients.

SOLUTION: This apoptosis-inducing agent has a synergistically enhanced apoptosis-inducing effect as compared with the case of using the TNF- α or IL-4 singly and can be used as an anticancer agent, a chronic rheumatoid arthritis-treating agent, an autoimmune disease-treating agent and a treating agent for hepatic diseases such as hepatitis, hepatic cirrhosis, and exhibiting less adverse effects.

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CLAIMS

[Claim(s)]

[Claim 1] The apoptosis inducer which makes TNF-alpha and IL-4 an active principle.

[Claim 2] The apoptosis inducer according to claim 1 which is a malignant tumor, or prevention and the remedy of rheumatoid arthritis.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]

[Field of the Invention] This invention relates to an apoptosis inducer.

[Description of the Prior Art] Apoptosis is one gestalt of the programmed cell death, and is contrasted with classic cell death (necrosis). Apoptosis happens to the bottom of the condition of the versatility on physiology. As the morphological description Condensation and pyknosis of chromatin relevant to lack of contact into a surrounding cell, the inspissation of cytoplasm, and the activity of endonuclease. Nuclear segmentation, disappearance of the microvillus of cell surface, smoothening (blistering) of cell surface; membrane blebbing) of cell surface, and fragmentation of DNA by ENDONUCLEASE are observed, it is discussed as a device in which the englobement is carried out by the cell which the last fragment of an APOTIKUS somatic cell adjoins (Duvall, E. and Wylie, A.H., Immunology Today, and 7 (4) --- 115-119(1986); Science, 245,301-305 (1989).

[0003] Although apoptosis is physiological cell death indispensable to normal generating and differentiation and it has happened to each cell in the cell kinetic of a normal body tissue etc., in diseases, such as a malignant tumor, leukemia, a growth sex skin disease, rheumatoid arthritis, and an autoimmune disease, apoptosis is controlled superfluously, consequently it is thought that a functional disorder arises into a cell. For example, WATANABE-FUKUNAGA and others is in the Fas molecule which participates in apoptosis about abnormalities in a MRL/lpr/lpr mouse, and the negative selection (apoptosis) device of the self-reactivity T cell in a thymus gland did not operate well, but it is suggested that the symptoms of an autoimmune disease develop as a result (Watanabe-Fukunaga, R., et al., Nature, 356,314-317 (1992)), moreover --- the process in which the chronic hepatitis shifts to liver cirrhosis and hepatic carcinoma --- apoptosis --- a control condition --- it is --- this --- a site --- an ibis --- it is thought that it progresses to the fibrosis and the liver cirrhosis following the inflammation of the hepatocyte by the cholic T cell. Therefore, the matter which guides the apoptosis of a cell which participates in this disease is useful as prevention and a remedy of the disease concerned.

[0004] The actinomycin-D which is the cycloheximide and inhibitor of RNA synthesis which are the former and a protein synthesis inhibitor, it reports that cytokine, such as a tumor necrosis factor (henceforth "TNF-alpha") and lymphotoxin (LT), has an induction operation of apoptosis --- having (Martin, S.J., et al., J.Immunol., 145, and 1859-1867 (1990) ---) Strelow, A., et al., J.Exp.Med., 192, 601-611 (2000). Recently, moreover, to interleukin 4 (henceforth "IL-4") it is reported that there is an apoptosis induction operation to Homo sapiens monocyte or eosinophile leucocyte (J. --- Immunol. and 148 (6) ---) 1812-1816 (1992), J.Allergy Clin.Immunol., 102 (6 Pt 1), 1013-1020 (1999).

[0005] However, the matter known until now was not enough from the point of apoptosis induction activity or a side effect, and the apoptosis inducer with high safety with high and apoptosis induction activity was called for.

[Problem(s) to be Solved by the Invention] This invention aims to let effectiveness offer a high

apoptosis inducer with high safety.

[0007]

[Means for Solving the Problem] The apoptosis inductive effect which each has to an undifferentiated cell or a precursor cell was reinforced in multiplication, and this inventions completed a header and this invention for it being useful as prevention, and a remedy of the disease accompanying superfluously control of apoptosis, when IL-4 were used together with TNF-alpha, as a result of inquiring wholeheartedly in view of this actual condition about the matter which has apoptosis induction activity.

[0008] That is, this invention offers the apoptosis inducer which makes TNF-alpha and IL-4 an active principle.

[0009]

[Embodiment of the Invention] Although the apoptosis inducer of this invention makes TNF-alpha and IL-4 an active principle With TNF-alpha, focusing on the living body defense mechanism through inflammation here Antitumor action, An osteoclasia operation, the incorporation inhibitory action of the lipid to a cell, a production induction operation of interleukin 1 and a colony stimulating factor, etc., It is the polypeptide of molecular-weight 17kDa which shows various bioactive, and is a kind of the cytokine which has antitumor action, an l-beam allergy induction operation, anti-inflammatory activity, etc. focusing on the immunocyte stimulation (differentiation to the plasma cell of a B cell, differentiation growth of a T cell) of the large range in IL-4. As mentioned above, it is reported to these TNF-alpha and IL-4 that there is an apoptosis induction operation, but when IL-4 are used together with TNF-alpha, this apoptosis inductive effect's being reinforced in multiplication is that completely predicting became impossible.

[0010] any of the recombinant produced by the natural mold or gene recombination which has the activity as TNF-alpha and IL-4, respectively as TNF-alpha used for the apoptosis inducer of this invention, and IL-4 --- although --- it is included.

[0011] TNF-alpha of a natural mold can be obtained by refining according to the known approaches, such as affinity chromatography and HPLC, from the culture supernatant of the existing cell strains, such as Sendai Virus (Sendai Virus) stimulus Homo sapiens B lymphoblast stock BALL-1, and TNF-alpha obtained by recombination of a gene can be obtained by refining similarly the Escherichia coli and the production protein of the existing cell strain which introduced the plasmid or vector incorporating a known gene.

[0012] Moreover, by mitogen etc., a Homo sapiens T cell clone, a peripheral blood T cell, or the existing cell strain of arbitration is refined similarly, and can be acquired from un-stimulating or the stimulated culture supernatant, and natural IL-4 can obtain recombination IL-4 as well as the above by use of the existing gene.

[0013] The apoptosis inducer of this invention may be prepared so that TNF-alpha and IL-4 may be contained in single pharmaceutical preparation, or it may prepare TNF-alpha and each of IL-4 as separate pharmaceutical preparation, and may use these two pharmaceutical preparation together. Moreover, as for TNF-alpha and the rate of a compounding ratio of IL-4, it is desirable not to be limited especially if the synergistic effect of an apoptosis induction operation can be demonstrated, but to especially be mixed in 1 - 99% of range, respectively, to blend TNF-alpha and to blend IL-4 at 70 - 30% 30 to 70%.

[0014] Thus, the prepared apoptosis inducer of this invention demonstrates the synergism that apoptosis inductive effect is reinforced remarkably, compared with the case where TNF-alpha or IL-4 are independently used as shown in the after-mentioned example. Therefore, compared with the case where TNF-alpha or IL-4 are independently prescribed for the patient, both dose can be decreased sharply, and it becomes mitigable [a side effect].

[0015] Although the dose per [to the adult of the apoptosis inducer of this invention] day is chosen suitably broadly, it is usually 50microg/body per day - 50 mg/body extent about TNF-alpha, and it is desirable about IL-4 to consider as 50microg/body per day - 50 mg/body extent.

[0016] The apoptosis inducer of this invention is used according to that purpose of use with various kinds of administration gestalten commonly used in this field as physic pharmaceutical preparation. This pharmaceutical preparation is prepared using a diluent or excipients, such as

as C mold, A mold, B mold, and a female mold, an Alzheimer disease, the Alzheimer mold senility Alzheimer's disease, Myocarditis, ARDS (adult respiratory urgency syndrome), an infectious disease, liver cirrhosis, prostatomegaly. It can be adapted also for various diseases, such as fibroid, bronchial asthma, arteriosclerosis, various congenital malformation, a nephritis, senile cataract, chronic fatigue syndrome (Chronic Fatigue Syndrome), and myotonia dystonica (Myotonic dystrophy).

[0023] When using this invention apoptosis inducer as an anticancer agent especially, apoptosis can be guided to a cancer cell by the administration, a carcinostatic operation is demonstrated, but if this is used together with various kinds of anticancer agents and radiotherapy which are known as a chemotherapeutic drug of cancer, the carcinostatic effectiveness can be promoted further and mitigation of a side effect can also be aimed at. As this chemotherapeutic drug, 5-fluorouracil (5-FU, consonance fermentation industrial incorporated company make), a mitomycin (Mitomycin-C, shrine make same as the above), futrafur (FT-207, Taiho Pharmaceutical, Inc. make), endoxan (Endoxan, Shionogi & Co., Ltd. make), a toyomycin (Toyomicin, Takeda Chemical Industries, Ltd. make), etc. are mentioned, for example.

[0024] [Example] An example is given to below and this invention is further explained to a detail. The monocyte was separated from an example 1(1) activity mold rheumatoid arthritis patient's bone marrow specimen by the concentration gradient method using the commercial kit (product made from Histopaque;Sigma). CD34 positivity cell (CD34+) was obtained from the separated monocyte using the magnetic bead (product made from Dynal CD34 progenitor cells selection system; Dynal). As for the cell which carried out separation recovery, the CD34+ cell of the CD19+ B cell was 0.5% or less at about 95%.

[0025] (2) Prepare by the culture medium which added SCF (10 ng/ml) and GM-CSF (1 ng/ml) so that it might become 1x10⁵ / well to a flat bottom microplate (No.3596;Costar) 24 well about a CD34+ cell. TNF-alpha (10 ng/ml) addition, IL-4 (10 ng/ml) addition, and its both (10ng/ml+10ng/ml) were cultivated for two weeks by addition or un-adding (control group). The cell was washed in PBS after culture, the cell was floated to PBS200microl containing 0.1% TritonX-100 and 0.1% sodium citrate, and the dyeing positivity cell (apoptosis dead cell) was measured with PI dyeing (10microl of 10microl/ml Propidium iodide is added, and it is 10 minutes at 4 degrees C) back flow cytometer (EPICS XL-Coulter). A result is shown in Table 1. In addition, the culture medium added and used penicillin G (100 unit/ml), streptomycin (100microl/ml), L-glutamine (0.3mg/ml), and FBS (10% product made from fetalbovine serum; Life Technologies) for RPMI-1640 culture medium (product made from Life Technologies). Moreover, the commercial item (product made from Pepro Tech EC) was used for each of SCF (stem cell factor), GM-CSF (granulocyte-macrophage colonystimulating factor), TNF-alpha, and IL-4.

[0026] [Table 1]

試験材料	死細胞率 (PI陽性細胞%)
対 照	2.33
TNF-α	2.06
IL-4	4.03
TNF-α / IL-4	10.7

[0027] From Table 1, it was shown that the rate of a dead cell reinforces in multiplication compared with the case where TNF-alpha or IL-4 are independently used for the apoptosis inducer of this invention which used TNF-alpha and IL-4 together.

[0028] TNF-alpha and the combined effect of IL-4 as well as an example 1 (2) were examined using the example 2Hela cell (uterine cervix squamous-cell-carcinoma origin Homo sapiens cultured cell). In addition, the Hela cell was cultivated on the plate 96 well in 2x104/a well. A result is shown in Table 2.

the bulking agent usually used, an extending agent, a binder, a *** agent, disintegrator, a surface active agent, and lubricant, as avirulent pharmacology support. Dosage forms can choose various kinds of gestalten according to the therapy purpose, and a tablet, a pill, powder, liquids and solutions, suspension, an emulsion, a granule, a capsule, suppositories, injections (liquids and solutions, suspension, etc.), ophthalmic solutions, etc. are mentioned as this typical thing. [0017] It faces fabricating in the gestalt of a tablet and a well-known thing can be conventionally used widely in this field as support. For example, a lactose, white soft sugar, a sodium chloride, grape sugar, a urea, starch, a calcium carbonate. Excipients, such as a kaolin, crystalline cellulose, and a silicic acid, water, ethanol, propanol, Simple syrup, grape-sugar liquid, starch liquid, a gelatin solution, a carboxymethyl cellulose, A shellac, methyl cellulose, potassium phosphate, the binder of polyvinyl-pyrrolidone sugar, Desiccation starch, sodium alginate, agar powder, the end of a laminaran. A sodium hydrogencarbonate, a calcium carbonate, and polyoxyethylene sorbitan fatty acid ester Sodium lauryl sulfate, a stearin acid monoglyceride, starch, Collapse inhibitors, such as disintegrator, such as a lactose, white soft sugar, stearin, cocoa butter, and hydrogenated oil. Absorption enhancers, such as a quarternary-ammonium-salt radical and sodium lauryl sulfate. Lubricant, such as a polyethylene glycol, etc. can be illustrated in adsorbents, such as moisturizers, such as a glycerol and starch, starch, a lactose, a kaolin, a bentonite, and a colloid silicic acid, purification talc, a stearate, and the end of a boric acid. Furthermore, a tablet can be used as the tablet which gave the usual coating if needed, for example, a sugar-coated tablet, a gelatin encapsulation lock, an enteric tablet, a film coated tablet or an auxiliary rim lock, and a multilayered tablet.

[0018] It can face fabricating in the gestalt of a pill, and a thing conventionally well-known in this field as support can be used widely, for example, disintegrator, such as binders, such as excipients, such as grape sugar, a lactose, starch, cacao butter, hardening vegetable oil, a kaolin, and talc, gummi arabicum pulveratum, powdered tragacanth, gelatin, and ethanol, and laminaran agar, etc. can be illustrated.

[0019] It can face fabricating in the gestalt of suppositories, and a conventionally well-known thing can be widely used as support, for example, the ester of a polyethylene glycol, cacao butter, higher alcohol, and higher alcohol, gelatin, semisynthetic glyceride, etc. can be mentioned.

[0020] When prepared as injections, liquids and solutions and suspension are sterilized, and it is desirable that they are blood and an isotonicity, and they can be faced fabricating in the gestalt of these liquids and solutions, an emulsion, and suspension, and can use all the things commonly used in this field as a diluent, for example, can mention water, ethyl alcohol, propylene glycol, ethoxylation isostearyl alcohol, polyoxy-ized isostearyl alcohol, and polyoxyethylene sorbitan fatty acid ester. In addition, the salt, the grape sugar, or the glycerol of sufficient amount to prepare an isosmotic solution in this case may be made to contain in physis pharmaceutical preparation, and the usual solubilizing agent, a buffer, an aponia-ized agent, etc. may be added.

[0021] Furthermore, a coloring agent, a preservative, perfume, a flavor agent, a sweetening agent, etc. and other drugs may be made to contain if needed in this invention apoptosis inducer. [0022] The apoptosis inducer of this invention obtained in this way can be applied to the various diseases resulting from superfluous control of apoptosis based on an apoptosis induction operation, and can expect the desired pharmacology effectiveness. As this application disease, for example Cancer, AIDS, ARC (AIDS associated diseases), ATL (adult T-cell leukemia; Adult T-cell leukemia), Hair Mr. cellularly leukemia (Hairy cell leukemia), the myelosis (HAM/TSP), HTLV-I associated diseases, such as respiratory disorder (HAB/HABA), arthrosis (HAAP), and uveitis (HAU), Collagen diseases, such as an autoimmune disease (systemic lupus erythematosus), for example, SLE, and rheumatoid arthritis (RA), Ulcerative colitis, Sjogren's syndrome, primary biliary liver cirrhosis, an outbreak thrombocytopenic purpura (Idiopathic Thrombocytopenic PurapuralTP), Autoimmune hemolytic anemia, myasthenia gravis, Hashimoto's disease, insulin-dependent (I-beam) diabetes mellitus, etc. can be illustrated. The apoptosis inducer of this invention Moreover, myelodysplastic syndromes, periodicity thrombocytopenia. Various kinds of diseases accompanied by thrombocytopenia, such as aplastic anemia, outbreak thrombocytopenia, and disseminated intravascular coagulation. Various kinds of hepatitis, such

[0029]

[Table 2]

試験材料	死細胞率 (P-I 陽性細胞) (%)			
	2 日目		3 日目	
	SCF/CM-CSF 無添加	SCF/CM-CSF 添加	SCF/CM-CSF 無添加	SCF/CM-CSF 添加
対 照	7.3	11.5	6.3	6.9
TNF-α	10.2	15.0	9.3	9.4
IL-4	10.1	10.3	8.0	8.3
TNF-α/IL-4	18.1	23.1	14.3	18.8

[0030] From Table 2, it was shown that the rate of a dead cell reinforces in multiplication compared with the case where TNF-alpha or IL-4 are independently used for the apoptosis inducer of this invention which used TNF-alpha and IL-4 together.

[0031]

[Effect of the Invention] Compared with the case where TNF-alpha or IL-4 are used independently, apoptosis inductive effect is reinforced in multiplication and can use this invention apoptosis inducer as liver disease therapy agents, such as an anticancer agent with few side effects, a rheumatoid arthritis therapy agent, an autoimmune disease therapy agent, hepatitis, and liver cirrhosis, etc.

[Translation done.]